# Relationship between ATP hydrolysis and molecular dynamics in co-freeze-dried sugar-ATP mixtures

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## INTRODUCTION

Freeze-drying is a manufacturing technique, used for stabilizing drugs that are otherwise unstable in solution. In some freeze-dried formulations, the active ingredient is present in low concentration and excipients, such disaccharides, are used variously as bulking agents during freeze-drying and for lyoprotection during the storage of freeze-dried materials. Above the glass transition temperature ($T_g$) co-operative and long range molecular dynamics underpin chemical and physical changes in the material, such as devitrification (the physical transition from a structurally disordered solid to a crystalline solid) causing loss of lyoprotective function.

For an amorphous formulation, the Shelf life determination from data from accelerated temperature above $T_g$ is not reliable for estimation of shelf life below $T_g$. An alternative approach is therefore required. The quantitative correlation between the molecular mobility (dynamics of the matrix) and the storage stability (Shelf life) is not well established.

## AIM

The aim of this work is to establish the relationships between sub-$T_g$ dynamics of the disaccharides and the differential levels of lyoprotection afforded to a moisture sensitive model drug substance (ATP).

## MATERIALS AND METHODS

3mL aliquots of solutions of 1% w/w ATP with 10% w/w sugar (either trehalose, maltose, or lactose) were freeze-dried in 10 mL glass vials by freezing at -40°C for 2 h; primary drying at -30°C for 60 h; and secondary drying at 20°C for 10 h. Moisture contents of the freeze-dried mixtures (~1.5%) were determined by TGA. Initial HPLC and BDS analysis were also performed after freeze-drying and after storage at 40°C for 30 days.

An ion pair reverse phase HPLC method was used [1] with an Agilent Eclipse C18 column with the detector set at wavelength 260nm. For identifying initial concentration of ATP at day zero, HPLC analysis was conducted from day 0 (initial) to day 90 for each co-freeze-dried sugar-ATP formulation.

Isothermal broad band dielectric spectra were recorded between 0.1 Hz and 1 MHz by placing ~300mg freeze-dried sample between two gold-coated electrodes of 25 mm diameter and 1 mm separation, at day 0.

## RESULT AND DISCUSSION

The hydrolysis of ATP to ADP and AMP is shown in Figure 1 for the three disaccharides.

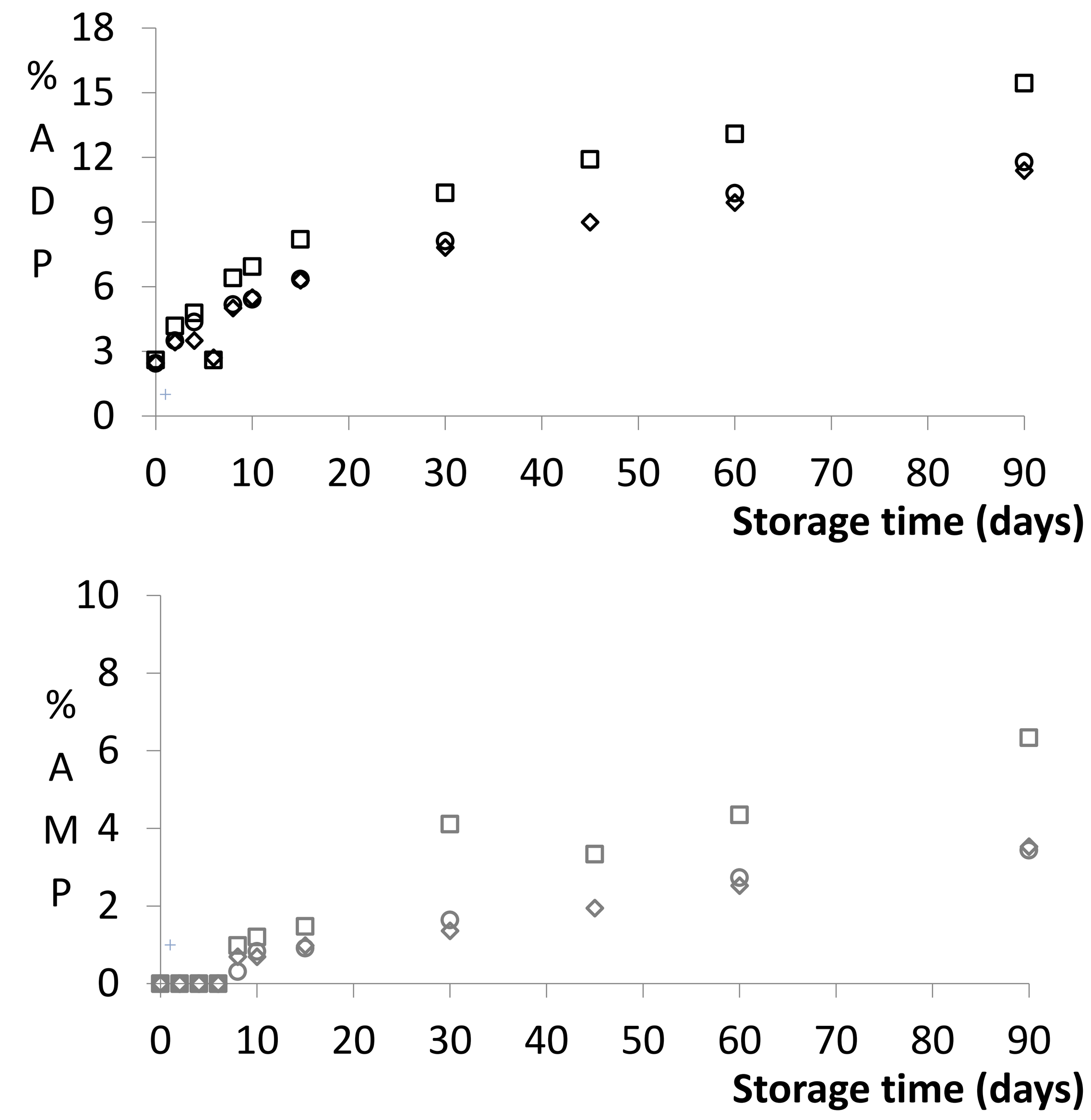


**Figure 1** detection of % ADP (black) and % AMP on storage of co-freeze-dried ATP with (disaccharides) lactose (◇), maltose (○), and trehalose (□)

The degradation profiles for lactose and maltose are similar and yet the moisture content for maltose (0.5%) was significantly lower than that for lactose (~1.5%) suggesting that lactose is a more effective lyoprotector in this case. The degradation profile for the trehalose stabilised ATP formulation shows greater hydrolysis than both lactose and maltose. While that may not be surprising in the case of maltose (given that maltose has a lower moisture content than trehalose at 1.7%) it is possibility unexpected when compared to lactose (given that lactose has a similar, albeit slightly lower moisture content to trehalose). This may suggest that latose is also a better lyoprotector than trehalose. These observations imply that the moisture content is not a single factor responsible in ATP hydrolysis and other factors such as the activity/mobility of water, and the packaging/molecular arrangement of the disaccharide must play important part in hydrolysis of ATP.

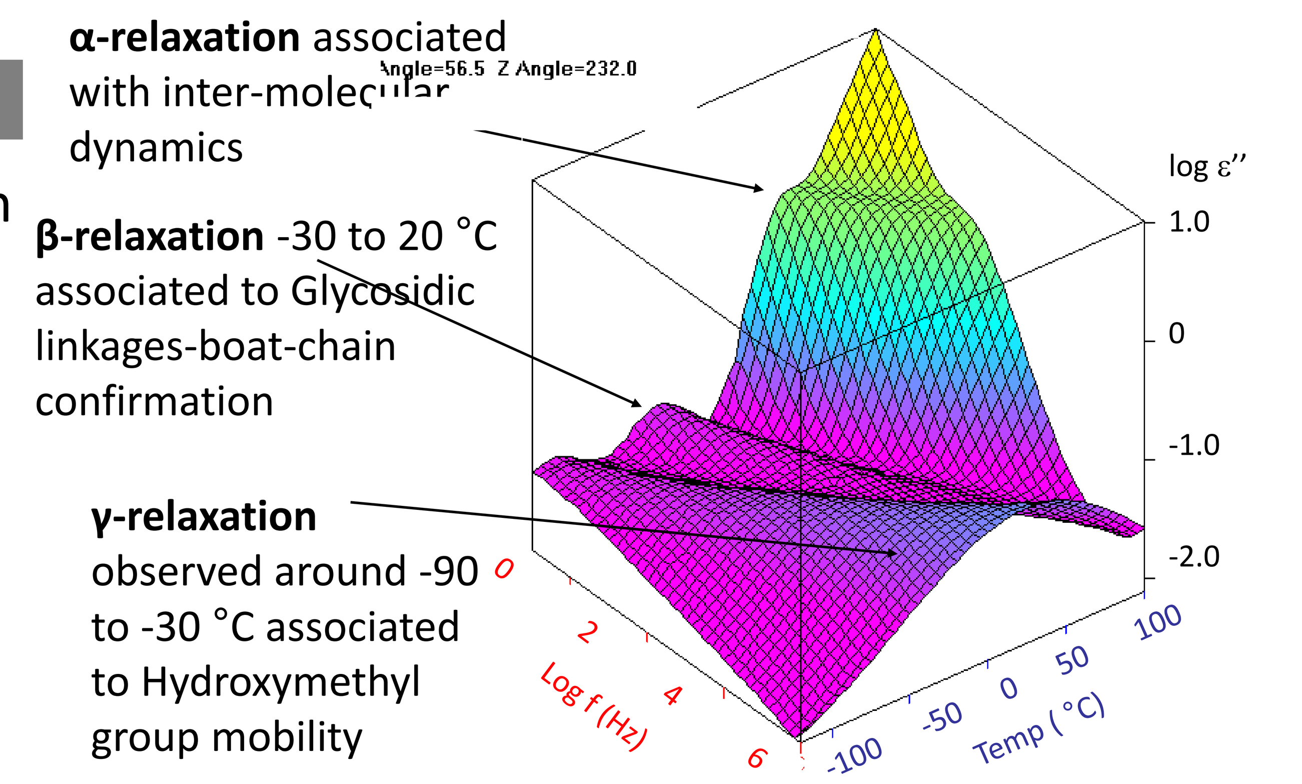


**Figure 2** Dielectric loss spectra of freeze dried Trehalose (+ 1% ATP 2.4 % moisture content).

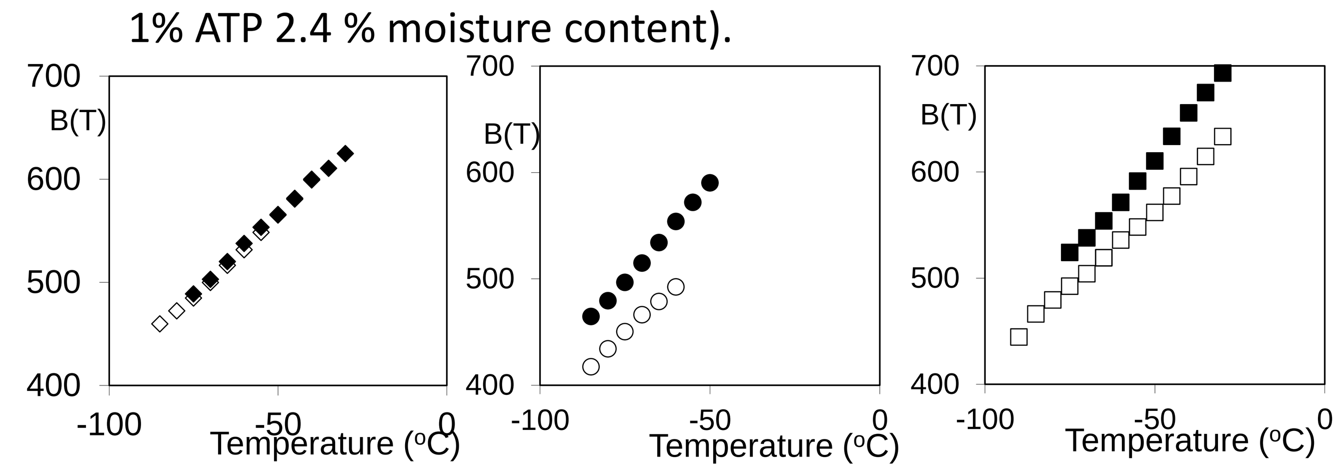


**Figure 3** Fröhlich parameter B (T) for dielectric γ-relaxation of lactose (◇), maltose (○) and trehalose (□) measured at a range of temperatures with % moisture content in range of 1.8-2.2 %.

In BDS analysis, the disaccharide-ATP formulations revealed two sub-$T_g$ processes (Fig 2 β and γ) and a α-relaxation process. Fig.3 highlights the differences in the magnitude of the Fröhlich parameter B(T) for the γ relaxation at day 0 and post storage at 40°C.

The marked increase in B(T) on storage at 40 C, for both trehalose and maltose, suggests a change in organization structure with an associated increase in the degrees of freedom which may correlate with the lyoprotection afforded by these sugars relative to lactose. The concept here is that mobility of matrix is coupled with mobility/reactivity of the water. This is consistent with the observation that the B(T) parameter for lactose doesn't change on storage, again suggesting that the lactose matrix is stable and therefore offers an enhanced degree of lyoprotection .

## CONCLUSION

The differential stability of ATP afforded by each disaccharide matrix correlates with dielectric relaxation process observed for the side chain hydroxymethyl group. The local-scale molecular mobility temperatures below $T_g$ may provide a specific opportunity for predicting stability in such systems.

## REFERENCE

1] S. Giannattasio Brain Res Protocols : 168

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